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Invention: PHARMACEUTICAL COMPOSITION HAVING ANTITUMOR ACTIVITY AND PROCESS FOR THE PREPARATION THEREOF

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SPECIFICATION

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Pharmaceutical composition having antitumor activity and process for the preparation thereof

Field of the invention

The present invention relates to a pharmaceutical composition having antitumor activity which contains herb medicines as the main ingredients, and process for the preparation thereof.

Prior arts

The inventor had invented a pharmaceutical composition of herb medicines having antitumor activity and process for the preparation thereof, and the invention was granted the Korean patent No. 72982.

The above-mentioned patent discloses a pharmaceutical composition containing Pulsatillae Radix (Pulsatilla koreana Nakai, P. cernua, P. danurica, P. ratensis, Chinese Pulsatillae, Mongolian Pulsatillae) and/or Clematis Chinensis Osbeclo (so called, Chinese clematis) as the main ingredients, and optionally Ulmaceae Cortex, Armeniacae Semen, Ginseng Radix and Glycyrrhizae Radix and process for the preparation thereof.

Pulsatillae species are grown wild all over the world, and the Pulsatillae Radix has been used as an antiphlogistic agent, astringent and hemostatic agent and thus for the treatment of dysentery in Korea. It is known that the Pulsatillae Radix contains anemonin, protoanemonin and saponin. Protoanemonin is the precursor of anemonin, and both may be dissolved in water, alcohol, chloroform, methylene chloride and the like.

Clematis Radix contains anemonin, anemonol and saponin. It has been used as an agent for gout, diuretic agent and agent for difficult menstruum.

Ulmaceae Cortex contains mucin and tannin, and has been used as a lenitive and adhesive.

Ginseng Radix has been known from ancient times as a marvellous medicine in the Far East. It has been used as a tonic, agent for acute gastritis and agent for various bleeding diseases. Recently, it is reported that Ginseng Radix has antitumor activity and contains Ginseng alkaloids, Ginseng saponins, essence oil, etc.

Glycyrrhizae Radix contains glycyrrhizin, liquiritin, licoricidin and liquiritoside and has been used as a cough remedy, expectorant, diaphoretic and agent for gastritis.

The above invention by the present inventor relates to a pharmaceutical composition having excellent antitumor activity and containing extract or powder of Pulsatillae Radix and/or Clematis Radix as the main ingredients, and optionally extract or powder of Ulmaceae Cortex, Armeniacae Semen, Ginseng Radix and Glycyrrhizae Radix.

By the method of the prior invention, the composition may be prepared by drying and finely powdering each herb ingredients ; by extracting the herb ingredients in a solvent selected from water, lower alcohol, chloroform, methylene chloride and the others which may dissolve the effective ingredients of the herbs at the temperature of 0℃ - the boiling point of the used solvent for 30 minutes to 24 hours and then vaporizing the used solvent to give the extract ; or dissolving said extract in water, alcohol or the mixed solvent thereof. When extracting the effective ingredients, each herb may be extracted independently or

two or more herbs may be extracted together. Then, the extract is powdered and formulated to a pharmaceutical preparation by using vehicles such as lactose, various starches, sucrose, mannitol, sorbitol and inorganic salts such as calcium phosphate, calcium sulfate, aluminium silicate and calcium carbonate ; binders such as sucrose, glucose, starch, gelatin, carboxymethylcelluose, methylcellulose, gum arabic, gum tragacanth, ethylcellulose, sodium alginate, hydroxypropylmethylcellulose, polyvinylpyrrolidone and soluble cellulose ; disintegrators such as starch, carboxymethylcellulose, methylcellulose and crystalline cellulose ; lubricants such as magnesium stearate and calcium stearate ; wetting agents such as glycerine, propylene glycol and sorbitol ; preservatives such as sodium benzoate, methyl p hydroxybenzoate, propyl p hydroxybenzoate, benzalkonium chloride, chlorobutanol and sodium dehydroacetate ; dissolving agents such as soluble alcohols and derivatives thereof, and various surfactants ; antioxidants such as sodium sulfite, sodium pyrosulfate, sodium metasulfate, sodium bisulfite, rongalite and ascorbic acid ; isotonic agents such as sodium chloride and dextrose ; indolent agents such as benzylalcohol and chlorobutanol ; and ointment bases such as vaseline, fluid paraffin, various vegetable oils, waxes and lanoline ; and other conventional auxiliary vehicles or carriers.

The inventor has continued intensive study to improve the antitumor composition and found that the composition by the prior invention is very unstable for preservation whereby it may easily lose its pharmaceutical effects in 3-6 months.

Summary of the invention

Accordingly, an object of the present invention is to provide an improved pharmaceutical composition which is stable and maintains its pharmaceutical efficacy even if it is preserved for several years, comprising lyophilized powder of Pulsatillae Radix, Ulmaceae Cortex, or mixture thereof as the main herb ingredients, and optionally one or more auxiliary herb ingredients selected from Ginseng Radix and Glycyrrhizae Radix, and conventional auxiliaries such as used in the prior invention (Korean patent No. 72982).

Particularly, the inventor has completed the present invention by discovering that herb ingredients should be extracted in a solvent at the temperature of below 60℃ and promptly lyophilized in order to maintain efficacy of the composition and preserve it for a long time.

Detailed description of the invention

The pharmaceutical composition in the present invention comprises as the main active herb ingredients 0-100wt% of Pulsatillae Radix and 0-100wt% of Ulmaceae Cortex, and optionally as the auxiliary herb ingredients 0-70wt% of Ginseng Radix and 0-70wt% of Glycyrrhizae Radix, wherein the contents are in terms of dried herb ingredients. Preferably the content of Pulsatillae Radix and/or Ulmaceae Cortex is over 30wt%.

The pharmaceutical composition having antitumor activity according to the present invention may be prepared by :

extracting powdered Pulsatillae Radix and/or powdered Ulmaceae cortex,

and optionally one ore more herb ingredients selected from powdered Ginseng Radix and powdered Glycyrrhizae Radix in a solvent at the temperature of below 60℃, filtering and lyophilizing the extract, and admixing the lyophilized powder with conventional auxiliaries, alternatively admixing the above extracted solution with auxiliaries, then filtering and lyophilizing the mixture, and then formulating the lyophilized powder to a pharmaceutical preparation by a conventional method used in the pharmaceutics.

In case the composition is to be used by injection, before lyophilization, it is advisable that the extracted solution is premixed with conventional auxiliaries including a preservative such as methylparaben, ethylparaben and propylparaben, an isotonic agent such as sodium chloride and an indolent agent such as benzylalcohol.

In case the composition is to be used in a form of capsule, tablet, ointment or the like except the injection, the extracted solution of the herb ingredients is lyophilized and then the lyophilized powder is admixed with conventional auxiliaries such as used in the invention of the Korean patent No. 72982 by a conventional method in the pharmaceutics to give a pharmaceutical preparation.

The solvent for extraction of herb ingredients may include water, lower alcohol, acetone, ethyl acetate, hexane and mixtures thereof.

The herb ingredients are extracted in a solvent at the temperature of below 60℃, and immediately lyophilized. The lyophilized powder of the herb ingredients as above may be filled into a vial and it may be

applied by adding distilled water for injection thereinto, or the lyophilized powder may be formulated to a form of capsule, tablet or ointment by a conventional method in the pharmaceutics.

About 100mg to 5g of the present composition on the basis of the lyophilized powder may be administered in a day, once a week to 1 - 3 times for a day. The dose of the composition may be varied in consideration of sex, age, condition of disease, etc. of the patients

The present invention will be explained in more detail with the following examples and experiments.

Comparative example 1

6.26g of powdered Pulsatillae Radix was added into 90ml of purified water and the mixture was warmed to 60℃ and stirred for 60 minutes, and then centrifuged at 3,500 rpm for 30 minutes. 50ml of the centrifuged solution was filtered in a sterilized room at below 60℃. The resulting solution was made to isotonic solution by adding NaCl under the aseptic condition, then sterile-filtered once again and divided to each 2.5 ml of the solution in an ampoule of 3ml under the aseptic condition, and sealed to obtain injection ampoules.

Comparative example 2

4g of powdered Pulsatillae Radix, 2g of powdered Ulmaceae cortex, 2g of powdered Ginseng Radix and 1g of powdered Glycyrrhizae Radix were added to 90ml of purified water and the mixture was stirred for 60 minutes at about 80℃ by adding purified water corresponding to the water distilled off. The resulting solution was cooled to room

temperature, centrifuged with 3,500 rpm for 30 minutes to obtain 46ml of the extracted solution. NaCl was added to the extract to obtain isotonic solution. The isotonic solution was filtered by a conventional method in a sterilized room, sterile-filtered and divided into each 2ml of the solution in an ampoule of 3ml, sealed and stored in a refrigerator.

Comparative example 3

62.6g of powdered Pulsatillae Radix, 31.3g of powdered Ginseng Radix and 10g of powdered Glycyrrhizae Radix were added to 900ml of purified water and extracted for 60 minutes at about 60℃ with adding purified water corresponding to the water distilled off. The resulting solution of 40ml was filtered and concentrated to give 26.4g of the concentrated extract.

Example 1

6g of powdered Pulsatillae Radix was added into 100ml of distilled water and extracted for 60 minutes at below 60℃ with stirring. After the extract was centrifuged with 5000 rpm, 900mg of NaCl as an isotonic agent and 160mg of methyl paraoxybenzoate were added thereto, and sterile-filtered in a sterilized room, divided into 20 vials of 5ml, promptly lyophilized at below -40℃ and sealed to obtain injectable powder.

Example 2

6g of powdered Pulsatillae Radix, 4g of powdered Ulmaceae cortex and 0.9g of powdered Glycyrrhizae Radix were added to 100ml of distilled water and extracted for 60 minutes at a temperature of below 60℃ with stirring. After the extracted solution was centrifuged with

5000 rpm, 900mg of NaCl as an isotonic agent and 160mg of methyl paraoxybenzoate as a preservative were added thereto. The resulting mixture was sterile-filtered in a sterilized room, divided into 20 vials of 5ml, promptly lyophilized at below -40℃, sealed to obtain injectable powder.

Example 3

6g of powdered Pulsatillae Radix, 3g of powdered Ginseng Radix and 0.9g of powdered Glycyrrhizae Radix were added to 100ml of distilled water and extracted for 60 minutes at the temperature of below 60℃ with stirring and with adding distilled water corresponding to the water distilled off. After the extracted solution was centrifuged with 5000 rpm, 900mg of NaCl as an isotonic agent and 160mg of propyl paraoxybenzoate as a preservative were added thereto. The resulting mixture was sterile-filtered in a sterilized room, divided into 20 vials of 5ml, promptly lyophilized at below -40℃, sealed to obtain injectable powder.

Example 4

60g of powdered Pulsatillae Radix, 40g of powdered Ulmaceae cortex and 9g of powdered Glycyrrhizae Radix were added to 1000ml of distilled water and extracted with stirring for 60 minutes at the temperature of below 60℃ with adding distilled water corresponding to the water distilled off. The extracted solution was centrifuged with 5000 rpm and promptly lyophilized at below -40℃ to give 38,150mg of the lyophilized powder.

Example 5

60g of powdered Pulsatillae Radix, 60g of powdered Ulmaceae cortex and 9g of powdered Glycyrrhizae Radix were added to 1000ml of 50%(v/v) ethanol and extracted for 60 minutes at the temperature of 50 - 60℃ with adding the alcohol corresponding to that distilled off. The extracted solution was centrifuged with 5000 rpm and promptly lyophilized at below -40℃ to give 45,150mg of the lyophilized powder.

Example 6

60g of powdered Pulsatillae Radix, 30g of powdered Ginseng Radix and 9g of powdered Glycyrrhizae Radix were added to 1000ml of 50%(v/v) aqueous solution of acetone and extracted for 60 minutes at the temperature of 50 - 60℃ with adding the solvent corresponding to the solvent distilled off. The extracted solution was centrifuged with 5000 rpm and promptly lyophilized at below -40℃ to obtain 34650 mg of the lyophilized powder.

Example 7

6g of powdered Pulsatillae Radix, 4g of powdered Ulmaceae cortex and 0.9g of powdered Glycyrrhizae Radix were added to 100ml of 70%(v/v) ethanol and extracted for 60 minutes at the temperature of below 60℃ with stirring and adding the solvent corresponding to the solvent distilled off. After the extracted solution was centrifuged with 5000 rpm, 900mg of NaCl as an isotonic agent and 160mg of methyl paraoxybenzoate as a preservative were added thereto. The resulting mixture was sterile filtered in a sterilized room, divided into 20 vials of 5ml, promptly lyophilized at below -40℃ and sealed to obtain injectable powder.

Example 8

10g of powdered Ulmaceae cortex was added to 100ml of 50%(v/v) ethanol and extracted for 60 minutes at the temperature of below 60℃ with stirring. The extracted solution was centrifuged with 5000 rpm, and 900mg of NaCl as an isotonic agent and thereto 160mg of methyl paraoxybenzoate as a preservative were added. The resulting mixture was sterile-filtered in a sterilized room, divided into 20 vials of 5ml, promptly lyophilized at below -40℃, sealed to obtain injectable powder.

Example 9

60g of powdered Pulsatillae Radix, 60g of powdered Ulmaceae cortex and 9g of powdered Glycyrrhizae Radix were added to 1000ml of hexane and extracted for 90 minutes at the temperature of below 60℃ with stirring and adding the hexane corresponding to the amount distilled off. The extracted solution was centrifuged with 5000 rpm and the resulting solution was promptly lyophilized at below -40℃ to obtain the lyophilized powder.

Example 10

Lyophilized extract obtained by the example 4	150mg
Crystalline cellulose	50mg
Lactose	50mg
Magnesium stearate	3mg

The above ingredients were cast into tablets by a conventional method and sealed with aluminium foil

Example 11

Lyophilized extract obtained by the example 6	150mg
Lactose	30mg
Corn starch	30mg
Talc	5mg
Magnesium stearate	3mg

The above ingredients were filled into a hard capsule of gelatin by a conventional method and sealed with aluminium foil

Example 12

Lyophilized extract obtained by the example 5	1000mg
Conventional ointment base	q.s.

The above ingredients were formulated into 10g of ointment, and filled and sealed into an aluminium tube.

Experiment 1

: Acute toxicity

The lyophilized powder obtained by the example 1 was administered to 8 rats of 234-276g, whereby the LD_{50} was 800mg/kg.

Experiment 2

: Antitumor effect

0.1ml of suspension of Sarcoma 180 cells($1x10^6$ cells) was injected (s.c.) into 30 rats of about 25g to develop tumors. After 6 days, 0.15ml

of injection prepared by dissolving the injectable powder of the example 1 in 5ml of distilled water for injection was injected (s.c.) to 10 rats once a day and 0.15ml of injection of the comparative example 1 was injected (s.c.) to another 10 rats once a day. While, 0.15ml of physiological saline solution was injected to the other 10 rats for 10 days as the control group.

Each 9 rats of the groups which were treated with the injection of the example 1 and the comparative example 1 were cured by injection for 15 days, and each 1 rat of the groups died at the 16th day, while 10 rats of the control group died all off beginning at the 10th day till to the 15th day.

Experiment 3

: Antitumor effect

0.1ml of suspension of Sarcoma 180 cells($1x10^6$ cells) was injected(s.c.) into 30 rats of about 25g to develop tumors. After 6 days, 0.15ml of injection prepared by dissolving the injectable powder of the example 1 in 5ml of distilled water for injection was injected (s.c.) to 10 rats (group 1) once a day, and 0.15ml of injection of the comparative example 1 preserved for 6 months in a refrigerator was injected (s.c.) to another 10 rats (group 2) once a day, and 0.15ml of physiological saline solution was injected to the other 10 rats for 10 days as the control group (group 3),

9 rats of the group 1 were cured with injection for 15 days, and 1 rat of the group 1 died at the 17th day. 3 rats of the group 2 were cured by injection for 15 days, 1 rat died at the 12th day, 3 rats died at the 15th day, and 3 rats were died at the 17th day. 10 rats of the group 3 died all off beginning at the 10th day till to the 15th day.

Experiment 4

: Antitumor effect

0.1ml of suspension of Sarcoma 180 cells($1x10^6$ cells) was injected (s.c.) into 48 rats of about 25g to develop tumors. Beginning at the 9th day(terminal stage of cancer), 0.15ml of sample injection prepared by dissolving the injectable powder of the example 3 in 5ml of distilled water for injection was injected (s.c.) to 7 rats once a day (group 1), each 0.15ml of the sample injection of the example 3 was injected (s.c.) to another 7 rats twice a day(group 2), 0.15ml of sample injection of the comparative example 1 was injected (s.c.) to another 7 rats once a day (group 3), each 0.15ml of sample injection of the comparative example 1 was injected (s.c.) to another 7 rats twice a day (group 4), 0.15ml of injection of the comparative example 1 which was preserved for 3 months in a refrigerator was injected (s.c.) to another 7 rats once a day (group 5), each 0.15ml of sample injection of the comparative example 1 which was preserved in a refrigerator for 6 months was injected (s.c.) to another 7 rats twice a day (group 6), and the other 6 rats were used as the control group.

In the group 1 and the group 3, each 1 rat died at the 15th day from carcinogenesis, each 1 rat died at the 17th day, each 1 rat died at the 18th day, and each 1 rat died at the 20th day, while each 3 rats were cured by injection for 20 days (at the 29th day from carcinogenesis).

In the group 2 and the group 4, each 1 rat died at the 16th day from carcinogenesis, each 1 rat died at the 18th day, each 1 rat died at the 19th day, and each 1 rat died at the 21th day, while each 3 rats were cured by injection for 19 days (at the 28th day from carcinogenesis).

In the group 5, 1 rat died at the 15th day of carcinogenesis, 1 rat

died at the 17th day, 1 rat died at the 18th day, 1 rat died at the 19th day, and 1 rat died at the 21th day, while 2 rats were cured by injection for 21 days.

In the group 6, 1 rat died at the 15th day of carcinogenesis, 1 rat died at the 16th day, 1 rat died at the 18th day, 1 rat died at the 19th day, and 2 rats died at the 20th day, while 1 rat was cured by injection for 20 days.

In the group 7, all rats died at the 15th day of carcinogenesis.

Experiment 5

: Clinical Test on a volunteer by administration of the injection prepared by dissolving the injectable powder of the example 1 in distilled water for injection.

Subject :

Name : KIM, Myung-Won (36 years old at the treatment, male)

Address : #6-501, Moran 2cha Apt., Shingi-dong, Dong-gu, Taegu, Korea

Kind of disease : Progressed thyroid cancer

Diagnosis : General hospital affiliated to the Youngnam University in Taegu on May 7, 1992.

Period of medication : From May 16, 1992 to October 20, 1992

Medication : 12ml of the injection was injected (i.v.) once a day for 4 days, while each 10ml of the injection was injected into the protruded tumor twice a day for 8 days (totally 15 times), and then no treatment of injection was performed for 25 days. After that, medication was repeated by i.v. injection and direct injection into the protruded tumor as the same method above, whereby the tumor disappeared

completely. After 5 years, the General hospital affiliated to the Youngnam University and the General hospital affiliated to the Chungnam University decided that the patient was completely cured.

Experiment 6

: Clinical Test on a volunteer by administration of the injection prepared by dissolving the injectable powder of the example 2 in distilled water for injection.

Subject :

Name : KIM, Chul-Ki (50 years old at the treatment, male)

Address : #1223-404, Mokdong Apt., Shinjung-dong, Yangchon-gu, Seoul, Korea

Kind of disease : Progressed lung cancer. Weight loss, Tussis, Bloody phlegm, Dyspnea due to residual cancer after operation.

Diagnosis : Christian Hospital in Wonju affiliated to Yonsei University on May 18, 1989.

Period of medication : From September 3, 1989 to March 5, 1990

Medication : 10ml of the injection was injected (i.v.) once a day for 4 days, and then no treatment was performed for 3 weeks. Same medication was repeated for 7 months of the above period, whereby the tumor and related symptoms disappeared completely. After 6 years, the General hospital affiliated to the Yonsei University and the General hospital affiliated to the Chungnam University decided that the patient was completely cured.

Experiment 7

: Clinical Test on a volunteer by administration of the injection prepared by dissolving the injectable powder of the example 2 in distilled water for injection.

Subject :

Name : SUH, Sang-Bong (35 years old at the treatment, male)

Address : 756, Shibang-ri, Jangmok-myeon, Geohje-gun, Kyungsangnam-do, Korea

Kind of disease : Progressed rectal cancer. Weight loss and severe pain due to residual cancer after partial removal of tumor by operation.

Diagnosis : Goshin Medical Center in Pusan on July 19, 1991.

Period of medication : From November 19, 1991 to May 12, 1992

Medication : 12ml of the injection was injected (i.v.) once a day for 4 days, and then no treatment was performed for 3 weeks. Same medication was repeated for 7 months of the above period, whereby the residual tumor disappeared completely. After 6 years from the medication, the Goshin Medical Center in Pusam and the General hospital affiliated to the Chungnam University decided that the patient was completely cured.

Experiment 8

: Clinical Test on a volunteer by administration of the injection prepared by dissolving the powder of the example 3 in distilled water for injection.

Subject :

Name : PARK, Ju-Sang (45 years old at the treatment, male)

Address : 15/2, 387-3, Bugok 1-dong, Kumjeong-gu, Pusan, Korea

Kind of disease : Progressed stomach cancer. Feeling heavy on the stomach, Dyspepsia and Weight loss.

Diagnosis : Goshin Medical Center in Pusan on December 29, 1989

Period of medication : From March 1, 1990 to September 29, 1990

Medication : 12ml of the injection was injected (i.v.) once a day for 4 days, and simultaneously each 173mg of the sample was taken internally four times a day. The i.v. injection was perform in such a manner that the injection was i.v. injected for 4 days and then no injection was made for 4 weeks, while the internal administration was continued. As the result, the symptoms disappeared completely and he was ascertained to be normal by the biopsy. After 6 years, the Goshin Medical Center decided that the patient was completely cured.

Experiment 9

: Clinical Test on a volunteer by administration of the injection prepared by dissolving the powder of the example 5 in distilled water for injection.

Subject :

Name : LEE, Bok-Do (65 years old at the treatment, male)

Address : 322-81, Chosan-ri, Hwagok-myeon, Yangsan-gun, Kyungsangbuk-do, Korea

Kind of disease : Progressed liver cancer. Abdominal dropsy, dyspepsia and weight loss due to residual cancer after partial removal of tumor by operation.

Diagnosis : Goshin Medical Center in Pusan on April 8, 1994.

Period of medication : From May 31, 1994 to December 30, 1994

Medication : 9ml of the injection was injected (i.v.) once a day for 4 days, and simultaneously 4ml of the sample was injected directly into the residual tumor in liver once a day for 3 days and then for 3 weeks no treatment was performed. The same medication was performed three times, and then from the 4th cycle of medication, only i.v. injection was performed, whereby the residual tumor disappeared completely. After 3 years, the Goshin Medical Center and the Hospital affiliated to Chungnam University decided that the patient was completely cured.

Stability test 1

Samples of the lyophilized power for injection prepared by the examples according to the present invention were preserved for 2 years and then dissolved in distilled water for injection. The resulting solution was transparent light brown and no precipitation settled.

Stability test 2

Samples of injection prepared by the comparative example 2 were stored for 1 month, 2 months and 3 months respectively. In the resulting solutions precipitate settled in 1 month and in three months the solutions was turbid whereby it was unable to be used for injection.

Effects of the invention

As seen from the above experiments, the antitumor compositions by the prior invention are sensitive to moisture, and even if stored in a refrigerator, they are easily deteriorated and efficacy thereof is severely decreased, whereby they cannot be preserved for a long time and then

they cannot be used as a medicine.

However, the present compositions which are prepared by extracting the herb ingredients at the temperature of below 60℃ and after extraction immediately lyophilizing the extract have no change in quality and efficacy thereof after long preservation, whereby they may be used safely.